174 HIV Integrase$^{R263A/K264A}$ Is Defective for TRN-SR2/IN Interaction and Nuclear Import of the PIC

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Background: Transportin-SR2 (TRN-SR2/TNPO3) is a human karyopherin encoded by the *TNPO3* gene. We identified TRN-SR2 as a binding partner of HIV-1 integrase (IN) and validated TRN-SR2 as an important cellular cofactor for the nuclear import of HIV-1. However, the question still remained whether the direct interaction between TRN-SR2 and IN mediates the nuclear import of HIV.

Methodology: By peptide screening we analyzed the interaction between TRN-SR2 and IN in molecular detail. Protein-protein interactions were verified by co-IP and AlphaScreen. We engineered site specific mutants in the C-terminal domain of HIV-1 IN that display reduced interaction with TRN-SR2 and studied their role in HIV replication using Q-PCR and fluorescence microscopy.

Results: Using the AlphaScreen protein-protein interaction assay we have been able to pinpoint the interacting hot spots in IN to R262/R263/K264 and K266/R269 in the IN C-terminal domain. We also identified a secondary interaction surface involving residues F185/K186/R187 and K188 in the catalytic core domain. Next, we introduced mutations at these positions in the C-terminal domain in the virus to corroborate the biological relevance of the interaction. Several mutations in the C-terminal domain of HIV IN inhibited the IN/TRN-SR2 interaction and rendered the virus replication-deficient. Some mutants affected reverse transcription (RT) compromising analysis of HIV nuclear import. All mutants also affected integrase activity. Still, one mutant, IN$^{R263A/K264A}$, retained full RT activity but displayed a specific block at the level of nuclear import as measured by Q-PCR and fluorescence microscopy. Although this mutant was defective for integration, no increase in 2-LTR circles was detected. Moreover, HIV encoding IN$^{R263A/K264A}$ showed reduced nuclear import when assayed with an eGFP-IN labeled HIV.

Conclusions: The IN$^{R263A/K264A}$ mutation in the C-terminal domain of HIV-1 integrase reduces the interaction with TRN-SR2 and specifically blocks HIV replication at the stage of nuclear import, corroborating the importance of this direct protein-protein interaction in HIV nuclear import.